GUANIDINE AND ITS RELATIONSHIP TO MUSCULAR DYSTROPHY

BY

ROBERT PRESTON MAC FATE

Ch.E., Lehigh University, 1925

M.S., University of Illinois, 1929

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHYSIOLOGICAL CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1941

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GUANIDINE AND ITS RELATIONSHIP TO MUSCULAR DYSTROPHY*

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THE abnormal metabolism of creatine (methylguanidine acetic acid) in progressive muscular dystrophy and certain other muscle conditions, as reported by Levene and Kristeller,[1] Brand and his co-workers,[2] Magee,[3] Thomas, Milhorat, and Techner,[4] Milhorat,[5] Tripoli and Beard,[6] and others, led me to study the effects of the administration of simple guanidine salts on creatine metabolism. It was first thought that administered guanidine might give rise to an added production and excretion of creatine. As the experiments progressed, the results suggested that the increased excretion of creatine following guanidine administration and in nutritional muscular dystrophy was due to changes produced in the muscle rather than to a direct production of creatine from the added guanidine.

MINIMUM LETHAL DOSE OF GUANIDINE HYDROCHLORIDE AND METHYLGUANIDINE SULFATE

Guanidine hydrochloride and methylguanidine sulfate were chosen for study. The minimum lethal doses of these compounds were determined to serve as a basis for their administration. The minimum lethal dose (M.L.D.) was defined as the number of milligrams of guanidine salt per gram of body weight which would cause the death within twenty-four hours of one or more of a series of fasting animals.

Guanidine hydrochloride and methylguanidine sulfate were administered intramuscularly and by stomach tube in a 5 per cent aqueous solution to healthy guinea pigs that had fasted twenty-four hours. The findings at the chosen end points are shown in Table I. Guinea pigs receiving the M.L.D. of either guanidine salt became nervous, showed varying degrees of trembling and twitching, and finally went into convulsions and died. Guinea pigs receiving less than the M.L.D. showed varying degrees of nervousness, trembling, and twitching, depending on the amount of guanidine salt given. Death occurred in some of the guinea pigs in this latter group, but later than twenty-four hours after the administration of the guanidine.

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Schmeideberg and Bunge[7] were among the first to show that benzoic acid is detoxicated in the kidney by conjugation with glycine and then excreted as the less toxic hippuric acid. Since that time it has been found that this synthesis takes place in other parts of the body as well as the kidney. It also has been found that the animal organism protects itself in the same manner from many toxic substances, such as phenylacetic acid, cresols, and others. Perhaps this same mechanism comes into play in the detoxication of simple guanidine salts.

Single and daily doses of 1 mg. of glycine per gram of body weight were found to be nontoxic in healthy guinea pigs. The protective action of this amount of glycine was then studied.

TABLE I

FATALITY OF THE M.L.D. OF GUANIDINE SALTS

GUINEA PIGS USED	SALT ADMINISTERED	METHOD OF ADMINISTRATION	M.L.D. (MG. PER GM. OF BODY WEIGHT)	ANIMALS DIED IN 24 HOURS (PER CENT)
14	Guanidine hydrochloride	Intramuscular	0.24	60
13	Guanidine hydrochloride	Oral	0.40	80
10	Methylguanidine sulfate	Intramuscular	0.29	75
10	Methylguanidine sulfate	Oral	0.50	50

TABLE II

PROTECTIVE ACTION OF SINGLE DOSES OF GLYCINE AND GLUCOSE AGAINST GUANIDINE HYDROCHLORIDE

TOTAL NUMBER OF GUINEA PIGS	GUANIDINE HYDROCHLORIDE	GLYCINE	GLUCOSE	NUMBER OF GUINEA PIGS SURVIVING	PER CENT SURVIVING	AVERAGE BLOOD GLUCOSE IN SURVIVORS, 6 HR. AFTER GUANIDINE (MG. PER 100 C.C.)
11	M.L.D.	No	No	3	27	54
12	M.L.D.	Yes	No	9	75	75
8	M.L.D.	No	Yes	3	38	65

Single doses of 1 mg. of glycine per gram of body weight were given by stomach tube to healthy guinea pigs that had fasted twenty-four hours. One hour later, the M.L.D. of guanidine hydrochloride was administered intramuscularly to 6 of the guinea pigs. The M.L.D. of methylguanidine sulfate was administered intramuscularly to the remaining 6 guinea pigs. All the guinea pigs showed the usual symptoms of guanidine intoxication and the majority died within twenty-four hours. Glycine in single doses, therefore, has no protective action against the M.L.D. of toxic guanidine salts.

The protective action of daily doses of glycine was then studied. Twenty-four guinea pigs were divided into two groups. Group A received daily doses of 1 mg. of glycine per gram of body weight. Group B received no glycine. On the second and following days, one-third M.L.D. of guanidine hydrochloride was administered intramuscularly to 4 guinea pigs of each group, one-third M.L.D. of methylguanidine sulfate was administered intramuscularly to an-

other 4 guinea pigs of each group, and one-third M.L.D. of methylguanidine sulfate was administered orally to the remaining 4 guinea pigs of each group. The amount of guanidine salt in each dose was uniformly increased at regular intervals until four-fifths M.L.D. was being given daily.

The average life of the guinea pigs receiving only the guanidine salts was twenty-eight days; the average life of those receiving glycine in addition was thirty-five days. On long-continued administration of guanidine salts, the guinea pigs appeared to develop a greater tolerance to the drug. However, the guinea pigs receiving glycine showed fewer and milder symptoms of guanidine intoxication and in addition lived longer. It appeared, therefore, that administered glycine aided the animals in overcoming the effects of toxic guanidine salts.

PROTECTIVE ACTION OF GLUCOSE

Watanabe[8] and others found that a severe hypoglycemia developed after the administration of toxic guanidine salts. Frank, Nothmann, and Wagner[9] found that the severity of spasms and the duration of life of animals poisoned by guanidine were dependent upon the amount of carbohydrate available in their organisms. Hummel[10] also noted this protective action of glucose, but Bakucz[11] was not convinced of its effect.

Experiments similar to those above were performed to study the protective action of glucose. The effects of single doses of glucose and of glycine against the M.L.D. of guanidine hydrochloride are shown in Table II. In experiments with daily administration, the average life of the guinea pigs receiving guanidine hydrochloride alone was eighteen days; of the guinea pigs receiving guanidine hydrochloride and glucose, nineteen days; and of the guinea pigs receiving guanidine hydrochloride and glycine, twenty-seven days. Glucose appeared to have no protective action.

EFFECT OF GLYCINE AND GUANIDINE HYDROCHLORIDE ON THE URINE AND MUSCLE CHEMISTRY OF NORMAL GUINEA PIGS

The urine and muscles of guinea pigs were analyzed to serve as controls for comparison with the work to follow on dystrophic guinea pigs. Healthy guinea pigs were placed in small metabolism cages and given a diet of carrots, grain, water, and orange juice. The urine was collected under toluene, measured and filtered daily. The muscles were obtained at death or on sacrificing the animal.

Urine was collected for a ten-day period to serve as a normal. Treatment was then given as shown in Table III. One milligram of glycine per gram of body weight was administered daily at 10 A.M. and 4 P.M. One-third M.L.D. of guanidine hydrochloride was administered at 5 P.M. The amount of guanidine salt in each dose was uniformly increased at regular intervals, as in previous experiments, until four-fifths M.L.D. was being administered daily.

The urines were analyzed for creatine and creatinine,[12] guanidine,[13] and total acid-soluble phosphorus.[14] The results were averaged for the normal period and for the period in which treatment was given. The fresh gastroc-

nemius and gluteus muscles were analyzed for creatine,[15] creatinine,[12] and the acid-soluble phosphorus fractions.[14, 16, 17] The results were averaged and are shown in Table III.

TABLE III

URINE AND MUSCLE CHEMISTRY FOLLOWING THE ADMINISTRATION OF GLYCINE AND GUANIDINE HYDROCHLORIDE TO NORMAL GUINEA PIGS

NO. OF GUINEA PIGS	4	4		4		4	
TREATMENT	NORMAL CONTROLS	NORMAL PERIOD	GLYCINE ADMINISTERED	NORMAL PERIOD	GUANIDINE ADMINISTERED	NORMAL PERIOD	GLYCINE AND GUANIDINE ADMINISTERED
Average days of treatment	42	10	32	10	26	10	32
Status at end of experiment	Alive		Alive		Dead		Dead
Average Urine Chemistry (mg. excreted per 100 Gm. of body weight per day)							
Creatine	1.5	1.5	1.9	1.6	1.8	1.6	2.1
Creatinine	2.9	3.0	3.0	3.1	3.0	3.1	3.1
Guanidine base	0.5	0.5	0.5	0.5	3.8	0.5	3.2
Total acid-soluble phosphorus	3.6	3.5	3.6	3.7	4.0	3.6	3.8
Per cent administered guanidine recovered	--	--	--	--	39	--	31
Average Muscle Chemistry (mg. per 100 Gm. of fresh tissue)							
Creatine	533	--	597	--	406	--	500
Creatinine	4.7	--	4.8	--	4.2	--	4.4
Acid-soluble phosphorus							
Total	182	--	161	--	116	--	146
Creatine phosphate	69	--	66	--	28	--	48
(per cent of total P)	(38)	--	(41)	--	(24)	--	(33)
Free orthophosphate	23	--	19	--	24	--	30
(per cent of total P)	(12)	--	(12)	--	(21)	--	(20)
Adenosine triphosphate	74	--	64	--	49	--	52
(per cent of total P)	(41)	--	(40)	--	(42)	--	(36)
Soluble esters	16	--	12	--	15	--	16
(per cent of total P)	(9)	--	(7)	--	(13)	--	(11)
Water content (%)	76.8	--	76.1	--	75.8	--	76.3

On the administration of glycine alone to normal guinea pigs, the excretion of creatine was increased, probably due to an increased production. An increased concentration of creatine was found in the muscles, although the relative per cent of creatine phosphate changed but little from the normal.

On the administration of guanidine hydrochloride alone to normal guinea pigs, an average of 39 per cent of the guanidine hydrochloride was recovered from the urine. The per cent of guanidine bases excreted decreased as the guanidine hydrochloride administered was increased. There may have been a maximum amount of guanidine that the kidney was capable of excreting or the decreased excretion may have been due to kidney damage. Histologic section of the kidneys showed some damage to the tubules. Some of the guani-

dine may have been converted to creatine, as was suggested by the slight increase in the excretion of creatine. It was more probable, however, that the guanidine hydrochloride either injured the muscle cell or made it more permeable, allowing an excessive loss of creatine to occur, as shown by the low concentration of creatine in the muscle. This loss may have stimulated the muscle or the centers of creatine formation to a greater production of creatine, with a subsequent further loss due to the abnormal permeability of the cell. This suggestion of an increased permeability was further supported by the loss of phosphates from the muscle and the decrease in the relative per cent of creatine phosphate.

On the administration of glycine with the guanidine hydrochloride, the excretion of creatine was increased, the effect being greater than when either glycine or guanidine hydrochloride were given alone. An average of 31 per cent of the guanidine hydrochloride administered was recovered from the urine. This was less than where the guanidine hydrochloride was given alone, and indicated that the glycine probably aided in the detoxication of the guanidine hydrochloride. This detoxication prevented the profound action of the guanidine hydrochloride on the muscle, as shown by the improved muscle metabolism and by the analysis of the muscle. The creatine and total phosphorus of the muscle and the relative per cent of creatine phosphate more nearly approached the normal.

EFFECT OF GLYCINE AND GUANIDINE HYDROCHLORIDE ON THE URINE AND MUSCLE CHEMISTRY OF GUINEA PIGS WITH NUTRITIONAL MUSCULAR DYSTROPHY

Goettsch and Pappenheimer[18] found that a diet composed of rolled oats, wheat bran, casein, lard, cod-liver oil, sodium chloride, and calcium carbonate, treated with ethereal ferric chloride, to which was then added skimmed milk powder, led to a progressive, highly selective and ultimately fatal dystrophy of the voluntary muscles. They found guinea pigs and rabbits to be susceptible and rats to be resistant. This diet is complete in known requirements except for vitamin E. However, Goettsch and Pappenheimer found that the addition of this factor did not prevent the development of the disease. The lesions were not due to inanition, infection, or scurvy, and must be referred to some still unknown factor.

A muscular dystrophy was developed in several litters of guinea pigs. Visible symptoms of muscular dystrophy were shown by the sluggish actions of the guinea pigs, difficulty in righting themselves when placed on their backs, inability to use their hind legs, and finally, inability to rise when placed on their sides. When definite symptoms of muscular dystrophy appeared, the weight had reached a plateau and in most cases was decreasing.

As previously noted, there occurs an abnormal metabolism of creatine in certain muscular dystrophies. Accordingly, the effects of glycine and toxic guanidine salts were studied in dystrophic guinea pigs.

Thirty-one dystrophic guinea pigs were divided into 4 groups. The dystrophy diet was continued. Group 1 was given no additional treatment. Group 2 was given 1 mg. of glycine per gram of body weight daily. Group 3 was given one-third M.L.D. of guanidine hydrochloride daily. Group 4 was given both

glycine and guanidine hydrochloride according to the amounts previously noted. The amount of guanidine salt in each dose was uniformly increased at regular intervals, as in previous experiments. The experiment was continued to the death of the guinea pigs. The chronologic data are shown in Table IV.

Twelve dystrophic guinea pigs were divided into 4 groups. They were placed in small metabolism cages, and the dystrophy diet and orange juice were continued. The urine was collected under toluene, measured and filtered daily. Urine was collected for seven days after dystrophy developed to serve as a control period. Treatment was then given these 4 groups as detailed for the preceding 4 groups.

TABLE IV

EFFECT OF GLYCINE AND GUANIDINE HYDROCHLORIDE ADMINISTERED TO GUINEA PIGS WITH NUTRITIONAL MUSCULAR DYSTROPHY

TREATMENT	NO. OF GUINEA PIGS	AGE AT START OF DYSTROPHY (DAYS)	AGE AT TIME OF MARKED DYSTROPHY (DAYS)	INTERVAL FROM START TO MARKED DYSTROPHY (DAYS)	AGE AT DEATH (DAYS)	INTERVAL FROM START OF DYSTROPHY TO DEATH (DAYS)
None	7	94	103	9	110	16
Glycine	8	95	106	11	116	21
Guanidine hydrochloride	8	93	98	5	103	10
Glycine and Guanidine hydrochloride	8	97	105	8	112	15

The urines were analyzed as already detailed, and the results were averaged for each period of the experiment. The fresh gastrocnemius and gluteus muscles were analyzed as detailed. The results were averaged and are shown in Table V.

The guinea pigs in which a nutritional muscular dystrophy was developed excreted a larger amount of creatine than did normal guinea pigs. This may have been due to defective storage, to degeneration of the muscle tissue, or to a greater production. It is probable that all three mechanisms were involved. Dystrophic guinea pigs also excreted a smaller amount of creatinine as the dystrophy developed, probably due to the decreased creatine metabolism. Guanidine excretion was increased, probably due to defective metabolism of these compounds. The production of toxic guanidine compounds in the muscle cell may have lowered the permeability of the cell, thus accounting for the loss of creatine from the muscle and its increased excretion. An initial larger excretion of phosphorus was followed by a decreased excretion, indicating a probable loss of phosphorus compounds from the muscle. These findings were confirmed by the muscle analysis.

Glycine appeared to have some effect in delaying the progress of nutritional muscular dystrophy. Clinically, the animals appeared more normal and their span of life was increased. An increased excretion of creatine was noted and an increased concentration was found in the muscle. This increased concentration of creatine was responsible, probably, for the better functioning of the muscle and for the delay in the progress of the dystrophy.

The toxic effects of guanidine hydrochloride were more pronounced in dystrophic guinea pigs than in the normal. Glycine appeared again to reduce

its toxicity. On the administration of guanidine hydrochloride to dystrophic guinea pigs, an average of 63 per cent of the guanidine hydrochloride was recovered from the urine. These figures were markedly higher than those obtained with normal guinea pigs, indicating a defect in the metabolism and detoxication of these substances.

TABLE V

URINE AND MUSCLE CHEMISTRY FOLLOWING THE ADMINISTRATION OF GLYCINE AND GUANIDINE HYDROCHLORIDE TO GUINEA PIGS WITH MUSCULAR DYSTROPHY

NO. OF GUINEA PIGS	3		3		3		3		12
TREATMENT	FIRST WEEK OF DYSTROPHY	BALANCE OF LIFE	FIRST WEEK OF DYSTROPHY	GLYCINE ADMINISTERED	FIRST WEEK OF DYSTROPHY	GUANIDINE ADMINISTERED	FIRST WEEK OF DYSTROPHY	GLYCINE AND GUANIDINE ADMINISTERED	AVERAGE OF ALL GUINEA PIGS FOR FIRST WEEK OF DYSTROPHY
Average days of treatment	7	9	7	14	7	5	7	11	7
Status at end of experiment		Dead		Dead		Dead		Dead	
Average Urine Chemistry (mg. excreted per 100 Gm. of body weight per day)									
Creatine	2.3	3.7	2.6	4.2	2.8	5.0	2.4	4.4	2.5
Creatinine	3.4	2.9	2.8	2.7	2.9	2.5	2.8	2.6	3.0
Guanidine base	0.5	0.6	0.6	0.6	0.5	3.9	0.6	4.8	0.6
Total acid-soluble phosphorus	3.7	3.0	3.5	3.1	3.0	2.3	3.1	2.9	3.3
Per cent administered guanidine recovered	–	–	–	–	–	63	–	53	–
Average Muscle Chemistry (mg. per 100 Gm. of fresh tissue)									
Creatine	–	206	–	248	–	151	–	190	–
Creatinine	–	1.8	–	2.1	–	1.6	–	2.0	–
Acid-soluble phosphorus									
Total	–	76	–	88	–	47	–	66	–
Creatine phosphate	–	23	–	32	–	11	–	20	–
(per cent of total P)	–	(30)	–	(36)	–	(23)	–	(30)	–
Free orthophosphate	–	15	–	11	–	12	–	12	–
(per cent of total P)	–	(20)	–	(13)	–	(26)	–	(18)	–
Adenosine triphosphate	–	29	–	35	–	16	–	25	–
(per cent of total P)	–	(38)	–	(40)	–	(34)	–	(38)	–
Soluble esters	–	9	–	10	–	8	–	9	–
(per cent of total P)	–	(12)	–	(11)	–	(17)	–	(14)	–
Water content (%)	–	76.0	–	76.4	–	75.7	–	76.1	–

EFFECT OF GLYCINE AND GUANIDINE HYDROCHLORIDE ON THE BLOOD, URINE, AND MUSCLE CHEMISTRY OF NORMAL DOGS

In the previous experiments the small size of the guinea pigs prevented extensive blood, urine, and muscle examinations. Dogs were adopted, therefore, as the test animal. Guanidine hydrochloride was used since it showed a greater toxicity than did methylguanidine sulfate. The compound was administered intramuscularly as it exerted a more profound effect in this manner.

The blood, urine, and muscles of 16 dogs were analyzed to serve as controls for comparison with the work to follow on dystrophic dogs. Healthy dogs were placed in metabolism cages and given the normal diet of meat and meal, with water ad lib. They were weighed twice a week. Urine was collected under toluene, measured and filtered daily. Blood was drawn twice a week. The muscles were obtained at death or on sacrificing the animal. Urine and blood were collected for a period of two weeks to serve as a normal. Treatment was then given as shown in Table VI.

One milligram of glycine per gram of body weight was administered daily by capsule. Eight-tenths milligram of guanidine hydrochloride per gram of body weight was injected intramuscularly one hour after the glycine. After three weeks the glycine and guanidine were increased to one and one-half times the original dose; after another two weeks, to two times the original dose; and after another two weeks, to two and one-half times the original dose. This latter dosage was continued to the death of the animals receiving guanidine hydrochloride.

The dogs receiving guanidine hydrochloride alone showed signs of guanidine intoxication after about the second week. They were nervous and showed trembling, which passed on to tetanic twitchings, convulsions, and death. The average life of these dogs was twenty-four days. The dogs receiving both glycine and guanidine hydrochloride were nervous and showed trembling and tetanic twitchings only two to three days before death. There were no other signs of intoxication over the period of the experiment. The average life of these dogs was forty-seven days.

The blood specimens were analyzed for sugar,[19] nonprotein nitrogen,[20, 21] creatine and creatinine,[22] amino acids,[23] guanidine,[24] inorganic and total acid-soluble phosphorus,[14] and lactic acid.[21, 25] The urines were analyzed for total nitrogen,[20, 21] creatine and creatinine,[12] guanidine,[13] and inorganic and total acid-soluble phosphorus.[14] The results were averaged for each period of the experiment. Table VI shows the average findings during the normal period and during the period of maximum dosage where the most striking changes were noted. Mixed samples of gastrocnemius, anterior tibialis, and biceps and quadriceps femoris were analyzed for creatine,[15] creatinine,[12] guanidine,[13, 26] acid-soluble phosphorus fractions,[14, 16, 17] sodium,[27, 28] potassium,[29] calcium,[27] chlorine by a modified silver nitrate and thiocyanate titration, and water content by drying to constant weight. The average results are shown in Table VI. Muscle and kidney specimens were examined histologically.

The muscles of the normal dogs and those receiving glycine were grossly and microscopically normal. The muscles of the dogs receiving guanidine hydrochloride alone showed many swollen and granular areas with a marked nuclear reaction. The muscles of the dogs receiving glycine and guanidine hydrochloride were normal grossly. Microscopically, a few swollen fibers were seen showing a marked nuclear reaction. The kidneys showed some damage to the tubules.

The oral administration of glycine to normal dogs produced an increased excretion of creatine. No other change was noted other than the increased blood amino acid concentration and an increased excretion of nitrogen which were to be expected.

TABLE VI

BLOOD, URINE, AND MUSCLE CHEMISTRY FOLLOWING THE ADMINISTRATION OF GLYCINE AND GUANIDINE HYDROCHLORIDE TO NORMAL DOGS

NO. OF DOGS	4	4		4		4	
TREATMENT	NORMAL CONTROLS	NORMAL PERIOD	GLYCINE ADMINISTERED	NORMAL PERIOD	GUANIDINE ADMINISTERED	NORMAL PERIOD	GLYCINE AND GUANIDINE ADMINISTERED
Average days of treatment	62	14	48	14	24	14	47
Status at end of experiment	Alive		Alive		Dead		Dead
Average Blood Chemistry (mg. per 100 c.c.)							
Sugar	84	82	85	86	66	84	75
Nonprotein nitrogen	28	26	27	29	48	28	54
Creatine	3.1	3.1	3.3	3.1	4.5	3.1	3.8
Creatinine	1.4	1.4	1.8	1.3	2.6	1.6	2.6
Amino acids	7.9	7.6	9.4	8.2	6.8	7.7	9.5
Guanidine base	0.4	0.3	0.3	0.4	2.5	0.4	2.9
Inorganic phosphorus	7.0	7.2	6.6	6.0	6.3	7.6	6.4
Total acid-soluble phosphorus	36	41	36	30	34	40	34
Lactic acid	17	17	18	16	37	17	29
Average Urine Chemistry (mg. excreted per 100 Gm. of body weight per day)							
Total nitrogen	38	36	48	39	45	37	46
Creatine	1.0	0.9	1.4	1.0	1.3	0.9	1.2
Creatinine	2.5	2.2	2.3	2.7	2.6	2.4	2.6
Guanidine base	0.6	0.6	0.5	0.6	3.3	0.6	2.8
Inorganic phosphorus	2.5	2.3	2.6	2.6	2.7	2.4	2.8
Total acid-soluble phosphorus	4.5	4.5	4.7	4.7	4.8	4.5	4.4
Per cent administered guanidine recovered	–	–	–	–	28	–	23
Average Muscle Chemistry (mg. per 100 Gm. of fresh tissue)							
Creatine	428	–	431	–	313	–	406
Creatinine	4.9	–	5.1	–	4.2	–	5.1
Guanidine base	0.7	–	0.7	–	2.4	–	1.8
Acid-soluble phosphorus							
Total	214	–	225	–	187	–	209
Creatine phosphate	87	–	92	–	42	–	72
(% of total P)	(41)	–	(41)	–	(23)	–	(34)
Free orthophosphate	28	–	32	–	49	–	35
(% of total P)	(13)	–	(14)	–	(26)	–	(17)
Adenosine triphosphate	88	–	90	–	68	–	81
(% of total P)	(41)	–	(40)	–	(36)	–	(39)
Soluble esters	11	–	11	–	28	–	21
(% of total P)	(5)	–	(5)	–	(15)	–	(10)
Sodium	43	–	40	–	82	–	57
Potassium	319	–	306	–	271	–	285
Calcium	8	–	9	–	6	–	8
Chlorine	62	–	60	–	114	–	77
Water content (%)	77.7	–	77.5	–	76.2	–	77.1

TABLE VII

BLOOD, URINE, AND MUSCLE CHEMISTRY FOLLOWING THE ADMINISTRATION OF GLYCINE AND GUANIDINE HYDROCHLORIDE TO DOGS WITH MUSCULAR DYSTROPHY

NO. OF DOGS	4			2			2			2			2
TREATMENT	DYSTROPHY CONTROLS			GLYCINE ADDED			GUANIDINE ADDED			GLYCINE AND GUANIDINE ADDED			WHEAT GERM ADDED
	DYST. DIET	DYST. DIET	DYST. DIET	DYST. DIET	PLUS GLYCINE	PLUS GLYCINE	DYST. DIET	PLUS GUANIDINE	PLUS GUANIDINE	DYST. DIET	PLUS GLYCINE AND GUANIDINE	PLUS GLYCINE AND GUANIDINE	DYST. DIET PLUS 10% WHEAT GERM
Clinical State	Norm.	Sl. dyst.	Mkd. dyst.	Norm.	Sl. dyst.	Mkd. dyst.	Norm.	Sl. dyst.	Mkd. dyst.	Norm.	Sl. dyst.	Mkd. dyst.	Norm.
Average days of treatment	161	7	19	168	26	21	154	3	10	159	12	18	215
Status at end of experiment			Dead			Dead			Dead			Dead	Alive
Average Blood Chemistry (mg. per 100 c.c.)													
Sugar	78	75	60	76	85	73	73	61	54	76	87	73	85
Nonprotein nitrogen	25	28	43	27	33	42	30	41	49	28	32	47	27
Creatine	3.0	3.0	2.3	3.0	3.2	2.8	2.9	3.5	3.8	3.0	3.4	2.8	3.1
Creatinine	1.5	1.1	1.0	1.6	1.7	1.3	1.5	1.8	1.7	1.7	2.0	2.0	1.4
Amino acids	9.5	9.7	8.3	8.5	9.5	8.7	9.2	8.4	8.1	8.7	9.7	8.8	8.9
Guanidine base	0.4	0.6	0.6	0.4	0.5	0.5	0.4	2.2	2.6	0.4	0.7	1.4	0.3
Inorganic phosphorus	8.1	7.7	8.8	8.8	8.8	8.0	9.0	8.1	7.7	7.6	8.4	7.6	8.4
Total acid-soluble phosphorus	56	54	60	50	55	48	48	49	41	50	55	50	49
Lactic acid	19	21	17	19	20	19	20	32	34	18	21	28	18

Average Urine Chemistry (mg. excreted per 100 Gm. of body weight per day)													
Total nitrogen	94	88	95	95	105	110	84	92	97	90	100	103	84
Creatine	1.5	1.6	2.4	1.2	1.6	2.0	1.1	1.4	1.7	1.0	1.1	1.7	1.1
Creatinine	2.2	1.9	1.7	2.3	2.7	3.2	2.4	2.2	1.8	2.1	2.4	2.9	2.5
Guanidine base	0.7	0.8	0.9	0.6	0.6	0.7	0.8	1.8	2.5	0.5	1.0	2.5	0.6
Inorganic phosphorus	2.4	2.8	3.1	2.2	2.8	2.8	2.7	2.5	2.2	3.0	2.9	3.0	2.8
Total acid-soluble phosphorus	5.1	5.3	4.1	4.8	5.4	5.3	5.5	5.5	5.0	5.8	6.3	6.3	5.3
Per cent administered guanidine recovered	–	–	–	–	–	–	–	48	59	–	40	48	–
Average Muscle Chemistry (mg. per 100 Gm. of fresh tissue)													
Creatine	–	–	311	–	–	343	–	–	263	–	–	332	395
Creatinine	–	–	3.4	–	–	3.7	–	–	3.1	–	–	3.7	4.7
Guanidine base	–	–	1.1	–	–	0.9	–	–	2.9	–	–	1.4	0.6
Acid-soluble phosphorus													
Total	–	–	144	–	–	152	–	–	126	–	–	140	192
Creatine phosphate	–	–	46	–	–	58	–	–	31	–	–	45	78
(% of total P)	–	–	(32)	–	–	(38)	–	–	(25)	–	–	(32)	(41)
Free orthophosphate	–	–	27	–	–	20	–	–	33	–	–	29	23
(% of total P)	–	–	(19)	–	–	(13)	–	–	(26)	–	–	(21)	(12)
Adenosine triphosphate	–	–	55	–	–	59	–	–	43	–	–	51	81
(% of total P)	–	–	(38)	–	–	(39)	–	–	(34)	–	–	(36)	(42)
Soluble esters	–	–	16	–	–	15	–	–	19	–	–	15	10
(% of total P)	–	–	(11)	–	–	(10)	–	–	(15)	–	–	(11)	(5)
Sodium	–	–	90	–	–	61	–	–	112	–	–	87	45
Potassium	–	–	237	–	–	280	–	–	197	–	–	244	323
Calcium	–	–	32	–	–	15	–	–	18	–	–	23	9
Chlorine	–	–	117	–	–	101	–	–	122	–	–	112	71
Water content (%)	–	–	76.1	–	–	76.7	–	–	75.8	–	–	76.0	77.5

The administration of guanidine hydrochloride to normal dogs produced an increased excretion of creatine. The greatest rate of excretion was found to occur in the first week or two of guanidine administration. It was probable, therefore, that the guanidine injured the muscle cell or lowered its permeability, allowing creatine to diffuse more rapidly than normal. The muscle was found to have a decreased creatine content, a decrease in the total acid-soluble phosphorus, and both a relative and an absolute decrease of creatine phosphate. A decrease in the blood sugar and an increase in the lactic acid showed a disturbance of the carbohydrate metabolism. An increased nitrogen retention was noted. A decrease in the potassium and an increase in the sodium and chlorine of the muscle showed a profound disturbance in the electrolyte balance. Guanidine bases were increased throughout, due to their administration.

The administration of glycine with the guanidine reduced the toxic action of the latter. The large supply of glycine aided directly in the detoxication and also promoted the production of creatine to replace that lost from the muscles. Metabolism in general appeared to be more normal. A nitrogen retention was still present, but less severe disturbances of carbohydrate metabolism and electrolyte balance were noted.

EFFECT OF GLYCINE AND GUANIDINE HYDROCHLORIDE ON THE BLOOD, URINE, AND MUSCLE CHEMISTRY OF DOGS WITH NUTRITIONAL MUSCULAR DYSTROPHY

A partial nutritional muscular dystrophy was developed in dogs. The dogs were weaned when they were forty-two days old and were placed in metabolism cages. The diet of Goettsch and Pappenheimer[18] was used. Sufficient water was added to the food to make a moist mass. This diet was supplemented by the use of additional skimmed milk powder dissolved in water, with the addition of vitamins A, C, and D. Water was allowed ad lib.

Morgulis and Spencer[30] found that the development of a nutritional muscular dystrophy following the diet of Goettsch and Pappenheimer could be prevented by the addition of whole wheat germ to the dystrophy-producing diet. Two dogs were placed on the diet with 10 per cent wheat germ added.

The dogs were weighed, and blood and urine were collected at two-week intervals during the first part of the experiment.

Visible symptoms of muscular dystrophy did not appear as rapidly nor was the dystrophy as pronounced as in the guinea pig. In about five months the weight had reached a plateau. Less interest was shown in food and the dogs appeared listless much of the time. A definite weakness finally appeared in the hind legs. Many of the animals died of pneumonia. These dogs were not included in the average findings given here.

When definite signs of weakness appeared in the hind legs, the dogs were weighed twice a week, urine was collected under toluene daily, blood was drawn twice a week, and the muscles were obtained at death or on sacrificing the animal. Treatment was then instituted as noted in Table VII.

The same amounts of glycine and guanidine were given as detailed above. After three weeks they were regularly increased as previously noted.

Blood and urine specimens were analyzed as previously discussed and the results were averaged for each period of the experiment. Table VII shows

the average findings for the dogs in each group during the period when they were on the dystrophy diet alone and appeared to be normal clinically; during the period of slight dystrophy, as shown by slight weakness; and during the period of marked dystrophy, as shown by marked weakness before death. The muscle specimens were analyzed as already described, and histologic sections were made. The results of the chemical analyses are shown in Table VII.

At autopsy the muscles of the thighs and hind legs of the dogs, which showed marked weakness before death, were found to be pale in color and flabby. Areas of damaged fibers were seen. Many were swollen, showing degeneration and a marked nuclear reaction. A moderate infiltration with fibrous tissue was seen. The muscles of the dogs receiving 10 per cent wheat germ were normal.

Little metabolic change was noted until the muscular dystrophy became evident clinically. A slight increase in the excretion of creatine was noted, seen more especially in the daily analyses than in the averages shown. When marked muscular dystrophy developed, a further increase was noted in the excretion of creatine, accompanied by an increased excretion of total acid-soluble phosphorus. A low blood creatine concentration finally developed and a low concentration of creatine and creatine phosphate were found in the muscles. An increased concentration of guanidine base was found in the blood, urine, and muscle. The increased concentration of guanidine in the muscle may have produced an increased permeability, with the resulting loss of creatine and phosphate. The increased guanidine was due, probably, to some defect in the metabolism of guanidine compounds. A disturbance also was seen in the carbohydrate and mineral metabolism, similar to that seen in the normal dogs when toxic guanidine compounds were injected intramuscularly.

Glycine appeared to delay the effects of the nutritional muscular dystrophy, as shown by the blood, urine, and muscle chemistry. The animals lived longer and their metabolism was more normal. The basis of this improvement may have been the increased concentration of creatine in the muscle due to its increased production.

Guanidine hydrochloride showed a greater toxicity in dogs suffering from a nutritional muscular dystrophy than it did in normal dogs. Glycine again aided in the detoxication of this added guanidine.

DISCUSSION

The administration of toxic guanidine salts over a period of several weeks results in a loss of creatine and phosphate from voluntary muscle. It appears probable that guanidine increases the permeability of the muscle cell, allowing creatine and phosphate to diffuse more rapidly than normal when they have been separated during muscular action. This suggestion is supported by the work of Gavrilescu,[31] who found by conductivity experiments that guanidine compounds increased the permeability of abdominal muscles. The lowered concentration of creatine in the muscle may stimulate the centers of creatine production, resulting in an increased production and further loss of creatine.

Increased blood guanidine and increased guanidine excretion have been noted in many conditions. Much confusion has resulted from the attempt to

associate various clinical symptoms with the level of blood guanidine. It is suggested that blood guanidine is the result, and not the cause, of endogenous guanidine intoxication. A guanidine derivative produced in any tissue may exert a more profound action in that tissue than it may exert after diffusion into the extracellular body fluids. The level of the blood guanidine probably depends on the activity of the centers of detoxication.

In exogenous guanidine intoxication other physiologic effects of guanidine may overshadow its action on the muscle cell. This effect is increased by the apparent ability of the animal to acclimate itself to larger amounts of administered guanidine over a period of time.

There is a similarity in the blood, urine, and muscle chemistry of guanidine intoxication and of nutritional muscular dystrophy. The increased concentration of simple guanidine compounds in nutritional muscular dystrophy suggests the possibility that these compounds may be the cause of the metabolic disturbance in this condition. The increased concentration of guanidine in the blood and urine of patients with progressive muscular dystrophy, as found by MacFate,[32] Sullivan, Hess, and Irrevere[33] and others, and the similarity of the muscle findings in this paper to the findings in progressive muscular dystrophy, as reported by Nevin,[17] Reinhold and Kingsley,[34] and others, suggest the possibility that toxic guanidine compounds may be responsible for, or at least initiate, the abnormal processes found in progressive muscular dystrophy and other dystrophies accompanied by like metabolic changes.

Hines and Knowlton[35] believe that the findings of altered concentration of various substances in atrophic muscles can be attributed to relative changes in the amounts of muscle cell and nonmuscle cell phases rather than to a change in the composition of the various phases. They found the increase in chloride concentration of atrophied muscle (from denervation, fasting, and tenotomy) to parallel the extent of atrophy and the relative increase in connective tissue, and, therefore, concluded that the chlorides of the muscle are chiefly associated with the connective tissue. Morgulis and Osheroff[36] state that at least a part of the chloride is a component of the connective tissues and that the increase in chloride content of dystrophic muscles is undoubtedly associated with the increase in fibrous tissue. The moderate increase in connective tissue noted in these experiments may account, in some degree, for the chemical changes observed. However, microscopic study did not reveal as great an increase in the connective tissue as is usually seen in cases of atrophy from denervation, fasting, and tenotomy. Further, because of the rapid and early chemical changes noted, it is believed that some other factor, such as an increased permeability of the muscle cell, must be considered.

The therapeutic value of glycine in these conditions probably lies in its stimulation of an increased production of creatine. This may be due to the specific dynamic action of the glycine itself (Rapport and Katz[37]). An increased glycine concentration in the blood and body fluids may stimulate the production of large amounts of creatine until the creatine production centers adjust themselves to this new and increased level of glycine. The rate of creatine production may then drop to approximately normal, accounting for the decreased excretion of creatine found in many patients after glycine therapy

over a long period of time, as reported by Thomas, Milhorat, and Techner,[4] Milhorat,[5] and others. The value of glycine in the treatment of progressive muscular dystrophy apparently depends upon the ability of the muscle cell to utilize the creatine produced. This ability appears to be lost gradually as the muscular dystrophy progresses.

The toxicity of guanidine derivatives varies greatly (Alles[38]). The general finding of increased blood guanidine cannot be properly evaluated until methods are perfected for determining the exact guanidine derivative present.

CONCLUSIONS

1. The daily administration of one-third to four-fifths M.L.D. of a toxic guanidine salt, over a period of several weeks, produces changes in the metabolism and structure of the muscle, similar to those found in nutritional muscular dystrophy and in progressive muscular dystrophy. There are produced a loss of creatine and phosphate from the muscle and severe disturbances in carbohydrate metabolism and the electrolyte balance. It is suggested that guanidine may lower the permeability of the muscle cell, producing these changes.

2. One milligram of glycine per gram of body weight, administered daily, appears to afford some protection against small daily doses (one-third to four-fifths M.L.D.) of a toxic guanidine salt.

3. Glycine delays the progress of a nutritional muscular dystrophy.

4. It is suggested that the abnormal metabolism of guanidine compounds may be an etiologic factor in the production of a progressive muscular dystrophy.

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Born in Duquesne, Pennsylvania, July 23, 1902. Attended the public schools of Duquesne, Pennsylvania, and Bethlehem, Pennsylvania, and was graduated from the latter in June, 1921 as Valedictorian. Received the Van Rensselaer Polytechnic Institute Gold Medal for perfection in mathematics and science, and a scholarship in Engineering from Lehigh University. Attended Lehigh University and was graduated, cum laude, in June, 1925. Received the degree of Master of Science in Physiological Chemistry in June, 1929, and the degree of Doctor of Philosophy in Physiological Chemistry in June, 1941, from the University of Illinois. Became a member of the American Chemical Society in 1924, and of the College of Medicine University of Illinois Chapter of Sigma Xi in 1928.

Appointments in the Research and Educational Hospitals, Chicago, Illinois: Hospital Chemist, 1925 to 1934. Assistant Director of the Hospital Laboratories, 1935 to date.

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